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Introduction

E.Z.N.A.® MicroElute Viral DNA/RNA Kit provides a rapid and easy method for the isolation of viral nucleic acid from plasma, serum, and other cell-free body fluids. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. The kit allows single or multiple, simultaneous processing of samples in under 20 minutes. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or ethanol, are eliminated. DNA purified using the E.Z.N.A® MicroElute Viral DNA/RNA method is ready for applications such as PCR, viral detection, and genotyping.

E.Z.N.A.® MicroElute Viral DNA/RNA Kit uses the reversible nucleic acid-binding properties of HiBind® matrix, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows viral nucleic acid bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® Viral spin columns to which nucleic acid binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality nucleic acid is finally eluted in sterile deionized water or low salt buffer.

Storage and Stability

All components of the E.Z.N.A.® Viral DNA/RNA Kit should be stored at 22°C-25°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. Under cool ambient conditions, a precipitate may form in the Buffer MSL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer MSL at room temperature.

Expiration Date: All E.Z.N.A.® Viral DNA/RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C.

Binding Capacity

Each HiBind ® Viral column can bind approximately 30 µg nucleic acid.

Kit Contents

Product No.	R6974-00	R6974-01	R6974-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ MicroElute column	5	50	200
2 ml Collection Tubes	15	150	600
Buffer TNA	5 ml	20 ml	60 ml
Buffer VHB	4 ml	20 ml	60 ml
Carrier RNA	150 ug	1 mg	1 mg
RWB Wash Buffer	2 ml	12 ml	4 x 12 ml
DEPC-Treated Water	2 ml	10 ml	10 ml
OB Protease	150 µl	1.5 ml	6 ml
User Manual	1	1	1

Before Starting

IMPORTANT	1	**RWB Wash Buffer** must be diluted with absolute ethanol (96-100%) as follows:	
		R6974-00	Add 8 ml absolute ethanol
		R6974-01/02	Add 48 ml absolute ethanol/per bottle
	2	**Carrier RNA** must be dissolved with DEPC Water before use and aliquot into adequate portions. Store at -20°C.	
		R6974-00	Add 150ul DEPC water
		R6974-01/02	Add 1 ml DEPC water
	3	**Buffer VHB** must be diluted with absolute ethanol:	
		R6974-00	Add 6 ml absolute ethanol
		R6974-01	Add 30 ml absolute ethanol
		R6974-02	Add 90 ml absolute ethanol

A. Spin Protocol: Purification of Viral Nucleic acid from Plasma, Serum or whole blood

Materials and equipments Supplied by User

- **Tabletop microcentrifuge and sterile 1.5 ml tubes.**
- **Water bath -** set to 37°C.
- **Ethanol -** approximately 0.3 ml per sample.

NOTE: The procedure below has been optimized for use with FRESH or FROZEN Plasma, Serum or blood samples from 1 to 200µl in volume. Other Cell-free samples can also be used. Bring samples and OB Protease solution to room temperature and have a water bath equilibrated to 37°C.**Carry out all centrifugation steps at room temperature**.

1. **Transfer 25 µl OB Protease into a sterile microcentrifuge tube.**

2. **Add 200 µl Plasmid or Serum into the tube and bring the volume up to 225 µl with DEPC treated water.**

3. **Add 225 µl Buffer TNA and 4 µl Carrier RNA. Mix thoroughly by vortexing.**

4. **Incubate sample at 45°C for 10 min.** Briefly vortex the tube once during incubation.

5. **Add 280 µl of isopropanol and mix thoroughly by vortexing.** Incubate at room temperature for 5 minutes.

6. **Assemble an HiBind® MicroElute Viral column in a 2 ml collection tube (provided). Transfer the lysate from step 5 into the column and centrifuge at 8,000 x g for 1 min to bind nucleic acid.** Discard the collection tube and flow-through liquid.

7. **Place the column into a second 2 ml tube (provided) and wash by pipetting 500 µl of Buffer VHB.** Centrifuge at 8,000 x g for 1 min. Again, Discard flow-through liquid and reuse the collection tube for next step.

8. **Place the column into a same 2 ml tube from step 7 and wash by pipetting 500 µl of RWB Wash Buffer diluted with ethanol.** Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

   Note that RWB Wash Buffer is provided as a concentrate and must be diluted

with absolute ethanol as indicated on the bottle or page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use.

9. **Using a new collection tube, wash the column with a second 400 µl of RWB Wash Buffer and centrifuge as above.** Discard flow-through and re-use the collection tube for next step.

10. **Place the empty column into the same 2 ml collection tube form step 9, centrifuge at maximum speed (15,000 x g) for 2 min to dry the column. This step is crucial for ensuring optimal elution in the following step.**

11. **Place the column into a sterile 1.5 ml microfuge tube and add 15-50 µl of DEPC-Treated water. Allow tubes to sit for 5 min at room temperature.**

12. **To elute nucleic acid from the column, centrifuge at 8,000 x g for 1 min.** Retain flow-through containing the nucleic acid. Place column into a second 1.5 ml tube.

B. Vacuum Protocol: Purification of Viral Nucleic acid from Plasma or Serum

Material and equipments supplied by user

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Vacuum Manifold**
- **Water bath -** set to 37°C
- **Ethanol -**approximately 0.3 ml per sample.

1. **Prepare the lysate by following step 1-5 of Protocol A, Spin protocol on page 4.**

2. **Insert the HiBind® MicroElute Viral Column into the vacuum manifold. Carefully apply the lysate to HiBind® MicroElute Viral column.** Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.

   Note: If the lysate has difficulty to pass through the column at this stage. Place the column into a collection tube (supplied). Close the lid and centrifuge at 8,000 x g for 5 minutes or until all liquid pass through the column. Place the column into another collection tube (supplied) and continue step 7 of the spin protocol.

3. **Pipet 500 µl of Buffer VHB into the column.** Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.

4. **Wash the column by pipetting 500 µl of RWB Wash Buffer diluted with ethanol into the column.** Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.

5. Close the lid of HiBind® MicroElute Viral column, remove it from the vacuum manifold. Insert the column into a collection tube (supplied) and centrifuge at 15,000 x g for 2 minute to completely dry the column.

6. Elute Nucleic acid as Step 11-12 on page 5.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Add the correct volume of Buffer MSL and incubate for specified time at 37°C. It may be necessary to extend incubation time by 20 min.
	Sample too large	If using more than 200 µl of samples, increase volumes of OB, Buffer MSL, and absolute ethanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 200 µl with DEPC Treated water.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at room temperature for 5 min with DEPC Treated water may increase yields.
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 5 before use.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or RT-PCR.
	Poor cell lysis due to incomplete mixing with Buffer MSL	Repeat the procedure, this time making sere to vortex the sample with Buffer MSL immediately and completely.
No Nucleic acid eluted	Poor cell lysis due to improper mixing with Buffer MSL.	Mix thoroughly with Buffer MSL prior to loading HiBind™ column.

Problem	Possible Cause	Suggestions
No nucleic acid eluted	Absolute ethanol not added to Buffer MSL.	Before applying sample to column, an aliquot of Buffer MSL/ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Washing leaves colored residue in column	Incomplete lysis due to improper mixing with Buffer MSL.	Buffer MSL is viscous and the sample must be mixed thoroughly.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Eluted material has red/brown color	Sample volume too large.	Reduce sample volume and follow directions